Interaction of the transforming acidic coiled-coil 1 (TACC1) protein with ch-TOG and GAS41/NuBI1 suggests multiple TACC1-containing protein complexes in human cells

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Dysregulation of the human transforming acidic coiled-coil (TACC) proteins is thought to be important in the evolution of breast cancer and multiple myeloma. However, the exact role of these proteins in the oncogenic process is currently unknown. Using the full-length TACC1 protein as bait to screen a human mammary epithelial cDNA library, we have identified two genes that are also amplified and overexpressed in tumours derived from different cellular origins. TACC1 interacts with the C-terminus of both the microtubule-associated colonic and hepatic tumour overexpressed (ch-TOG) protein, and the oncogenic transcription factor glioma amplified sequence 41/NuMA binding protein 1 (GAS41/NuBI1; where NuMA stands for nuclear mitotic apparatus protein 1). This suggests that the TACC proteins can form multiple complexes, dysregulation of which may be an important step during tumorigenesis.

Key words: breast cancer, glioma, transcription factors, microtubules.

INTRODUCTION

Tumorigenesis is a multistep process involving many genes. The accumulation of genetic changes, such as structural chromosomal abnormalities, is often associated with the increase in the malignant potential of cancer cells. Thus, in order to understand the complex events leading to the development and progression of cancer, it is necessary to identify and characterize candidate genes that may be involved in both the initiation and progression of the disease. Recently, we have identified a novel family of evolutionarily conserved genes, named the transforming acidic coiled-coil (TACC) genes, which are characterized by the presence of a large coiled-coil motif (the TACC domain) located at the C-terminus of each family member [1,2].

Several pieces of evidence implicate the TACC family in oncogenic processes. First, the human *TACC* genes are located in regions of the genome that are associated with cancer: the amplification or rearrangement of the chromosomal regions containing the *TACC1* and *TACC2* genes is implicated in breast tumour progression [1,3], while the third family member, *TACC3*, is located within 200 kb of a translocation breakpoint associated with multiple myeloma [2]. Secondly, *in vitro* and *in vivo* studies indicate that the TACC proteins are intimately linked to the processes of cell growth and differentiation. TACC1 and TACC3 are expressed at high levels during embryogenesis and are then down-regulated in differentiated tissues [1,2,4]. However, both TACC1 and TACC3 are expressed at high levels in human cancer cell lines [1,2]. TACC1 has the properties of a classic oncogene, in that it can transform mouse fibroblasts and promote anchorage-independent growth, which are characteristics shown by metastatic cancer cells [1]. Thus TACC1 and TACC3 are likely to be involved in the processes that promote cell division prior to the formation of differentiated tissues. Unlike TACC1 and TACC3, expression of the second member of this family, TACC2, is widespread in the adult (I. H. Still, B. Lauffart and O. Gangisetty, unpublished work); however, in a model for breast tumour progression, TACC2 is down-regulated as breast tumours become more malignant [5]. Furthermore, reintroduction of the partial TACC2 clone, anti-zuai-1 ('AZU-1'), into these breast tumour cells reduces the ability of these malignant cells to grow [5]. Thus TACC2 has the properties of a breast tumour suppressor gene. Interestingly, the transformation of mouse fibroblasts by the oncogene, Ha-Ras, leads to an increase in TACC2 mRNA levels, suggesting that induction of TACC2 may be important in oncogenic cell signalling events initiated by other known oncogenes [6].

To begin to understand the functional role of the TACC proteins, we previously investigated the normal subcellular distribution of the human TACC-1, -2 and -3 proteins [7]. During interphase, the TACC proteins are distributed throughout the cell, with TACC1 and TACC3 showing a preferential accumulation in the nuclei of cells examined. Interestingly, as predicted from the protein sequence, antibodies raised against the human TACC proteins also stain the mitotic spindle and the centrosomes in mitotic HeLa and primary fibroblast cells [7]. Recently, the TACC domain of the *Drosophila* TACC (D-TACC) protein was shown to bind to msps ('mini spindles'), the *Drosophila* homologue of the human colonic and hepatic tumour overexpressed (ch-TOG) protein [8,9]. This has led to the proposal that this interaction plays a role in the stabilization of centrosomal microtubules [8,9]. Murine TACC3 has also been shown to interact with the members of the arylhydrocarbon nuclear translocator (ARNT) family, and is also known as ARNT interacting protein (AINT) [4]. These transcription factors play critical roles in embryogenesis, cellular responses to chemical carcinogens and tumour progression/metastasis. Murine Tacc3 interacts with ARNT1 and can up-regulate ARNT-mediated responses to hypoxia and the carcinogen dioxin [4]. Thus it is still unclear whether the sole role of the TACC proteins is in the organization of the microtubule network by acting as

Abbreviations used: ARNT, arylhydrocarbon nuclear translocator; AINT, ARNT interacting protein; ch-TOG, colonic and hepatic tumour overexpressed; DAPI, 4,6-diamidino-2-phenylindole; NuMA, nuclear mitotic apparatus protein 1; GAS41/NuBI1, glioma amplified sequence 41/NuMA binding protein 1; GFP, green fluorescent protein; TACC, transforming acidic coiled-coil; D-TACC, *Drosophila* TACC; TACIP, TACC-interacting protein.

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adaptor molecules between non-microtubule proteins and microtubule-associated proteins, or whether the TACC proteins perform additional functions distinct from their association with microtubules. The identification of additional TACC-interacting proteins (TACIPs) is therefore likely to shed further light on the function of these proteins in normal and tumour cells.

We now present results of screening a yeast two-hybrid library for TACIPs. First, we demonstrate that TACC1 binds to the C-terminal section of the microtubule-associated human ch-TOG protein. In addition, we show that TACC1 interacts with the oncogenic transcription factor factor glioma amplified sequence 41/NuMA binding protein 1 (GAS41/NuBI1; where NuMA stands for nuclear mitotic apparatus protein 1). Both ch-TOG and GAS41/NuBI1 are amplified and up-regulated in cancers of different cellular origins. This suggests that the TACC proteins form multiple complexes, dysregulation of which is an important step during tumorigenesis.

MATERIALS AND METHODS

Yeast two-hybrid analysis

The Matchmaker yeast two-hybrid system and human mammary epithelial cDNA library were obtained from ClonTech Laboratories (Palo Alto, CA, U.S.A.). To construct the bait plasmid, pASTACC1, the TACC1 initiator start methionine was first replaced by a *Sal*I site using PCR directed site-directed mutagenesis. Subsequently, the TACC1 open reading frame and approx. 200 bp of the 3′ untranslated region was cloned into the GAL4 DNA-binding domain vector pAS2.1 (ClonTech Laboratories). This construct was transformed into yeast strain CG1945 using the lithium acetate method, and lack of autoactivation or non-specific interactions between the bait TACC1 plasmid and the GAL4 activation domain and lamin C negative control proteins indicated that the entire open reading frame could be used for screening. Library screening was carried out by the sequential transformation method according to the manufacturer's instructions. Clones that activated the *His3* and *LacZ* reporter genes only in the presence of the pASTACC1 plasmid were considered positive. The pACT2 plasmids containing cDNAs encoding potential TACIPs were isolated using the glass bead-phenol/chloroform method (Clontech Laboratories; Yeast Protocols Handbook PT3024-1) and transformed into chemically competent *Escherichia coli* cells (strain DH5-α; Invitrogen, Carlsbad, CA, U.S.A.). Clones were sequenced using an ABI sequencer, courtesy of the Cleveland Clinic Foundation DNA sequencing core facility.

Elucidation of protein binding domains of GAS41 and TACC1

Subclones of TACC1 and GAS41 were generated by PCR and cloned into pAS2.1 and pACT2 respectively (primer sequences are available on request). Clones were sequenced prior to transformation into yeast. pASTACC1 and subclones of TACC1 were transformed into the yeast strain Y187 (ClonTech Laboratories), and pACT2-GAS41 subclones were transformed into CG1945. Expression of each construct was confirmed by Western-blot analysis using a monoclonal antibody raised against the GAL4 domain of the fusion protein (results not shown). Interactions between bait and target plasmids were examined using mating assays, according to the manufacturer's protocols.

Generation of stable cell lines expressing green fluorescent protein (GFP)-tagged GAS41

GAS41 cDNA was amplified from human brain cDNA using gene specific primers GAS41IMET (5′-GGA**CAATTGG**GTTCAAGAGAATG-3′) and GAS41R2 (5′-TTT**CTCGAG**CTTACTACCAAG-3′). The amplified product was cloned into pCR2.1 (Invitrogen), and sequenced to confirm validity. The GAS41 insert was then isolated using *Mfe*I and *Xho*I (these sites are shown in bold in the primer sequences) and cloned into the *Eco*RI/*Sal*I site of the pEGFPC3 vector (ClonTech Laboratories). A stable cell line expressing EGGAS41 was then generated by transfecting pEGGAS41 into HEK-293 cells and the breast cancer cell line MDA-MB-468, as described previously [1]. Stable G418-resistant clones were examined by fluorescence microscopy, and EGGAS41 protein was detected by Western-blot analysis.

Immunological reagents

An immunogenic peptide, corresponding to residues 220–232 (PELVPSRRSKLRK; single-letter amino acid notation) of TACC1 was selected and generated by the multiple antigen peptide-synthesis method (Cleveland Clinic Foundation Biotechnology Core). This peptide was then used to produce a polyclonal antiserum to TACC1 in rabbits (performed by Rockland Immunochemicals, Boyertown, PA, U.S.A.). To confirm specificity, the antiserum was tested using immunohistochemical assays, and immunoprecipitation of GFP-tagged TACC1 with an antibody raised against GFP (#8372-2; ClonTech Laboratories), followed by Western-blot analysis, using the termination bleed antiserum (1:3000 dilution; results not shown). In native HEK-293 and MDA-MB-468 cells, the TACC1 antibody detects a protein of 110 kDa. Secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.).

Co-immunoprecipitations and Western-blot analysis

HEK-293 cells were washed with ice-cold PBS, prior to scraping off in RIPA buffer [50 mM Tris/HCl (pH 7.2, at 25 °C), 150 mM NaCl, 1 % (v/v) Triton X-100, 1 % (w/v) sodium deoxycholate, 0.1 % (w/v) SDS and a cocktail of protease inhibitors]. The cell extract was passed several times through a 21-gauge needle and centrifuged at 3500 ***g*** for 15 min at 4 °C. Following centrifugation, the supernatant was removed and 200–500 μg of extract was incubated with primary antibody (2 μg) for 1 h at 4 °C. Anti-rabbit IgG agarose conjugate (#A8914; Sigma, St Louis, MO, U.S.A.) was then added and immunoprecipitation was allowed to proceed for an additional 1 h at 4 °C. Immune complexes were pelleted by centrifugation (1000 ***g*** for 5 min at 4 °C) and washed three times with RIPA buffer, and immunoprecipitated proteins were eluted by boiling with 2 × Laemmli buffer [125 mM Tris/HCl (pH 6.8, at 25 °C), 4 % (w/v) SDS, 20 % (v/v) glycerol, 10 % (v/v) 2-mercaptoethanol and 0.004 % (w/v) Bromophenol Blue]. Cell lysates and eluted complexes were separated by SDS/PAGE (8 % gels) and transferred on to Immobilon membranes (Millipore, Marlborough, MA, U.S.A.). After blocking with TBS [10 mM Tris/HCl (pH 8, at 25 °C)/150 mM NaCl]/Tween 20 (0.02 %, v/v) containing 5 % (w/v) skimmed milk powder, the membrane was incubated with TACC-specific antisera in blocking buffer for 16 h at 4 °C. The membranes were washed with TBS/Tween 20 (0.02 %, v/v) and incubated for 1 h with horseradish peroxidase-conjugated secondary antibody. After washing, protein was visualized with the ECL® Plus detection system (Amersham Biosciences, Piscataway, NJ, U.S.A.).

Indirect immunofluorescence

HEK-293 and MDA-MB-468 cells were cultured on glass coverslips overnight in Dulbecco's modified Eagle's medium containing 10 % (v/v) fetal calf serum. Cells were fixed with 2 %

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(v/v) formaldehyde for 15 min, permeabilized with 0.2% Triton X-100 and then blocked with 10% (v/v) normal rabbit serum prior to incubation with the anti-TACC1 serum at 1:100 dilution for 1 h. The primary antibody was detected with a rhodamine-conjugated anti-rabbit antibody (Santa Cruz Biotechnology), and nuclei were counter-stained with 4,6-diamidino-2-phenylindole (DAPI). Cells were examined at 40× magnification. All procedures were carried out in the Roswell Park Cancer Institute Cell Analysis Facility.

RESULTS

Identification of TACIPs

To identify proteins that interact with TACC1, we fused the full-length TACC1 open reading frame to the GAL4 DNA-binding domain of the pAS2.1 vector. After confirming that this construct was unable to autoactivate the reporter genes in the CG1945 yeast host strain, this bait protein was used to screen an adult mammary epithelial cDNA library (ClonTech Laboratories). Approx. 10^6 transformants were plated and selected on His^- selective medium containing 10 mM 3-aminotriazole. Of the 69 His^+ clones originally isolated, 38 proved positive when assayed for β-galactosidase activity using the colony lift assay. Subsequent isolation and sequence analysis revealed that four of these clones corresponded to the C-terminal 529 amino acids of a previously identified TACC binding protein, the human ch-TOG protein (Figure 1A), and that five corresponded to the C-terminal region of the putative oncogenic transcription factor GAS41/NuBI1 (Figure 1B).

Mapping of binding domains on GAS41/NuBI1 and TACC1

TACIP38, the smallest of the GAS41/NuBI1 cDNA clones identified, corresponded to the C-terminal 109 amino acids of GAS41/NuBI1 (Figure 1B). The final 60 amino acids of this region is predicted to form an α-helical region, which has recently been shown to bind to the nuclear matrix protein and mitotic spindle component, NuMA [10]. To determine whether the TACC1 binding site on GAS41/NuBI1 overlapped with that for NuMA, we generated an additional smaller construct containing the C-terminal amino acids 168–227. This construct activated the *His3* and *LacZ* reporter genes in the diploid strain only in the presence of the pASTACC1 construct, demonstrating that the TACC1 binding occurs within the predicted NuMA binding domain (Figure 1B). This suggests that TACC1 and NuMA could compete for binding to GAS41/NuBI1.

To date, the C-terminal TACC domain has been shown to be important for the interaction of the TACC proteins with components of the microtubule network [8,9], and the ARNT transcription factors [4]. To determine whether the TACC domain also constituted the GAS41/NuBI1 interacting domain, we next sought to assay the ability of GAS41 to bind to a series of smaller TACC1 constructs. These constructs were expressed as GAL4 DNA-binding domain constructs in yeast strain Y187. TACIP38 (in yeast strain CG1945) was then mated to each construct and diploids were assayed for specific interaction with each region of TACC1. Figure 2(A) demonstrates that activation of the reporter genes was only observed with pASTACC1 constructs containing the region between amino acids 206–427. The region between amino acids 263–446 contains three highly acidic imperfect repeats of 33 amino acids, which, based upon their amino acid composition, we have termed SDP repeats (Figure 2B). Hence, the region spanning these repeats appears to be the major determinant for TACC1 binding to GAS41.

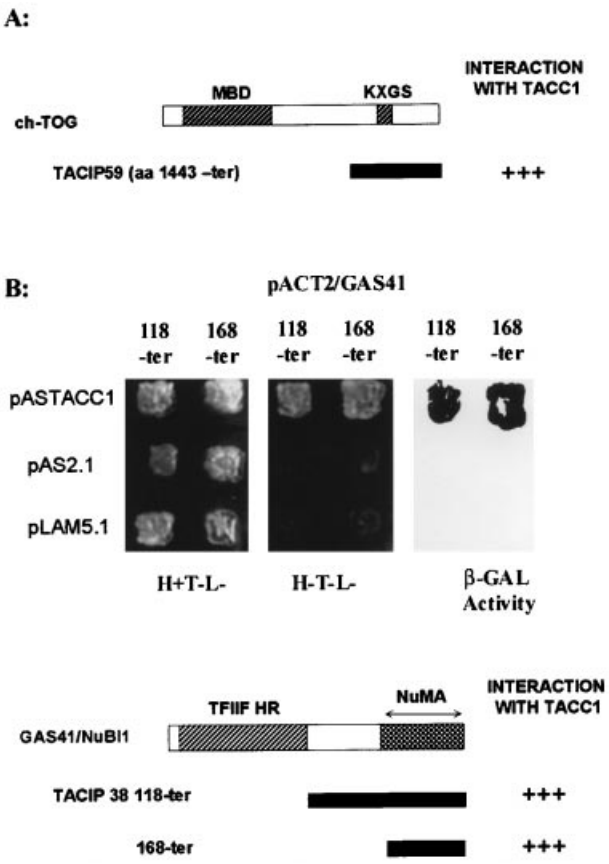

Figure 1 ***In vitro*** **interactions between TACC1 and potential TACIPs defined by yeast two-hybrid analysis**

(**A**) Yeast two-hybrid screening with the full-length TACC1 open reading frame identified four clones corresponding to amino acids 1443–C-terminus (-ter) of ch-TOG. This region contains the KXGS motif responsible for binding tubulin dimers, and is distinct from the microtubule binding domain (MBD). (**B**) Two GAL4-activation domain–GAS41 constructs, TACIP38 and GAS41 168-ter, were assayed for their ability to interact with the full-length TACC1 open reading frame (pASTACC1) using the yeast two-hybrid system. In mating assays, both constructs were able to activate the *His3* reporter, permitting growth on medium lacking histidine, and the *LacZ* gene [positive for β-galactosidase (β-GAL) activity], only in the presence of the TACC1 open reading frame. Neither construct activated the reporter genes in the presence of the pAS2.1 vector alone or the non-specific control pLAM5.1, expressing lamin C. The TACC1 binding site of GAS41/NuBI1 is located in the C-terminal α-helical domain, overlapping the NuMA binding site. The general transcription factor IIF homology region (TFIIF HR) occupies the N-terminal 117 amino acids of GAS41/NuBI1. H, histidine; T, threonine; L, leucine.

In vivo interaction between GAS41/NuBI1 and TACC1

To determine whether TACC1 and GAS41 associate *in vivo*, we first stably transfected HEK-293 cells with a cDNA encoding the GAS41/NuBI1 protein fused to the C-terminus of GFP (EGGAS41). Western-blot analysis of the stable cell line EGGASB1 confirmed that the GFP fusion product was correctly expressed (results not shown), and this clone was selected for further analysis. TACC1 specifically co-immunoprecipitated with the EGGAS41 fusion protein, using an antibody raised against the GFP moiety (Figure 3). This interaction was dependent on the presence of the GAS41 moiety, as GFP alone failed to co-immunoprecipitate with TACC1. Thus GAS41/NuBI1 and TACC1 are found in the same complex *in vivo*.

To confirm further that GAS41/NuBI1 and TACC proteins could physically interact in the intact cell, we determined whether GAS41/NuBI1 co-localized with endogenous TACC1 *in vivo*.

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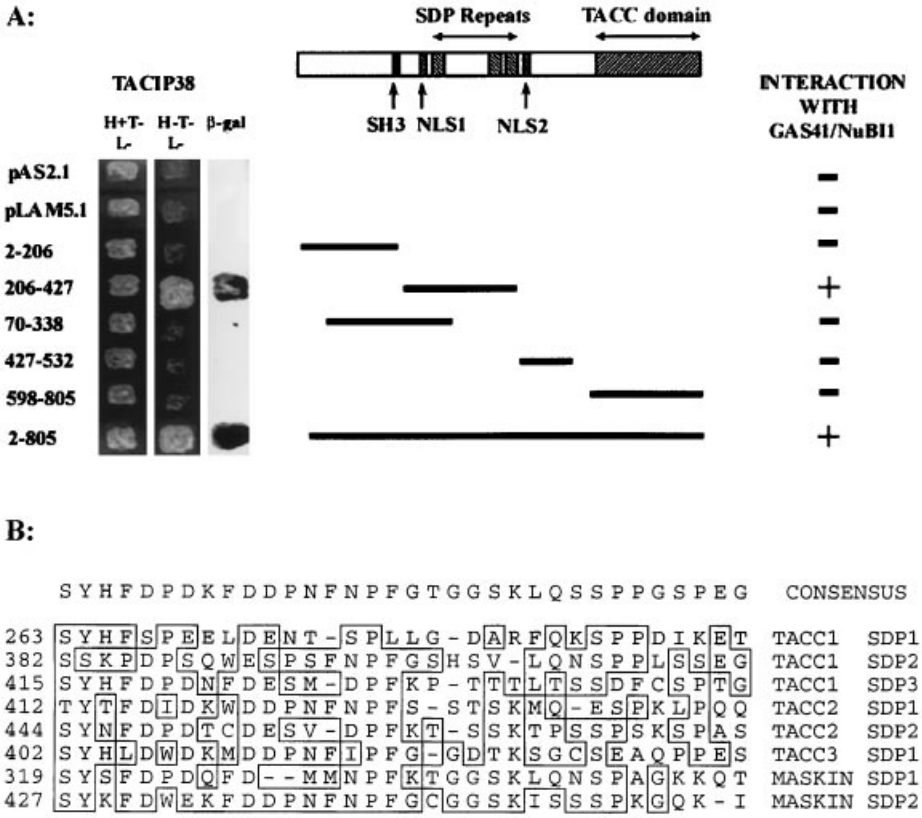

Figure 2 The GAS41 binding domain of TACC1 is located in the region containing the conserved SDP repeats

(**A**) To define further the binding domain for GAS41 on TACC1, a series of smaller TACC constructs were constructed and expressed as GAL4 DNA-binding domain constructs in yeast strain Y187. TACIP38 (in yeast strain CG1945) was mated to each construct and assayed for specific interaction with each region of TACC1. Interaction was only obtained with constructs containing amino acids 206–427, which contains the SDP repeats, and nuclear localization signal (NLS)1. SH3, Src homology 3. H, histidine; T, threonine; L, leucine; β-gal, β-galactosidase. (**B**) Sequence comparison of the SDP repeats from the TACC protein family. Each human TACC protein has a different number of these repeats. The *Xenopus* maskin protein also contains two SDP repeats, suggesting an evolutionary conserved function for the SDP repeat.

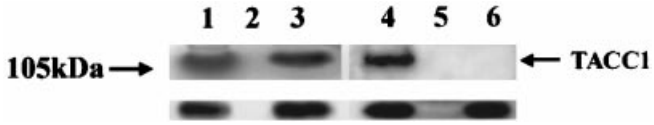

Figure 3 *In vivo* interactions between GAS41 and TACC1

Stable HEK-293 transfectants expressing either GFP fused to GAS41 (EGGASB1) or GFP alone (EGFP/HEK-293) were immunoprecipitated with either anti-GFP or rabbit IgG and immunoblotted with anti-TACC1 antibody. Due to its structure and acidic nature, TACC1 migrates at 110 kDa (compared with the predicted molecular mass of 88 kDa) in EGGASB1 and EGFP/HEK-293 cells (lanes 1 and 4 respectively) and is specifically immunoprecipitated from EGGASB1 cells by the anti-GFP antibody (lane 3), but not control IgG (lane 2). No interaction between GFP and TACC1 was detected in immunoprecipitates of EGFP/HEK-293 expressing GFP alone (lane 6). Lane 5 represents EGFP/HEK-293 immunoprecipitated with control IgG. Bottom panels confirm that similar amounts of GFP–GAS41 (53 kDa) and GFP (27 kDa) are immunoprecipitated by the anti-GFP antibody in lanes 3 and 6.

Stable transfection of the EGGAS41 plasmid into HEK-293 cells, and the breast cancer cell line MDA-MB-468 resulted in an accumulation of GFP staining in the nucleoplasm, but not the nucleoli of these cells (Figure 4). Indirect immunofluorescence microscopy using the TACC1 antibody revealed a similar staining pattern to the EGGAS41 fusion protein, together with some low-level diffuse staining in the cytoplasm. The nuclear accumulation of TACC1 in these transfected cell lines was not a side effect of the overexpression of the GFP fusion protein, as no difference in TACC1 localization was noted in HEK-293 and MDA-MB-468 cells transfected with GFP vector alone (Figure 4) or untransfected cells (results not shown). Therefore the primary site of interaction between GAS41 and TACC1 is likely to be the nuclei of interphase cells.

DISCUSSION

The evolutionarily conserved TACC family is comprised of proteins found in mammals [1,2,4], *Xenopus* [11] and *Drosophila* [12]. Originally, based upon their protein sequence, the human TACC proteins were predicted to play a role in nuclear scaffolding and/or mitotic spindle assembly [1]. These potential roles have been partly supported from studies of their subcellular localization [7]; however, further clues as to their functional roles will only come from the identification of potential TACIPs. We have now begun a systematic attempt to isolate these proteins by yeast two-hybrid analysis. Using this method, we have now demonstrated that TACC1 interacts with the microtubule-associated protein ch-TOG, and the oncogenic transcription factor/NuMA-binding protein GAS41/NuBI1. Expression analysis has revealed that TACC1 and TACC3 are expressed in several tissues prior to the onset of differentiation [1,2,4]. However, both TACC1 and

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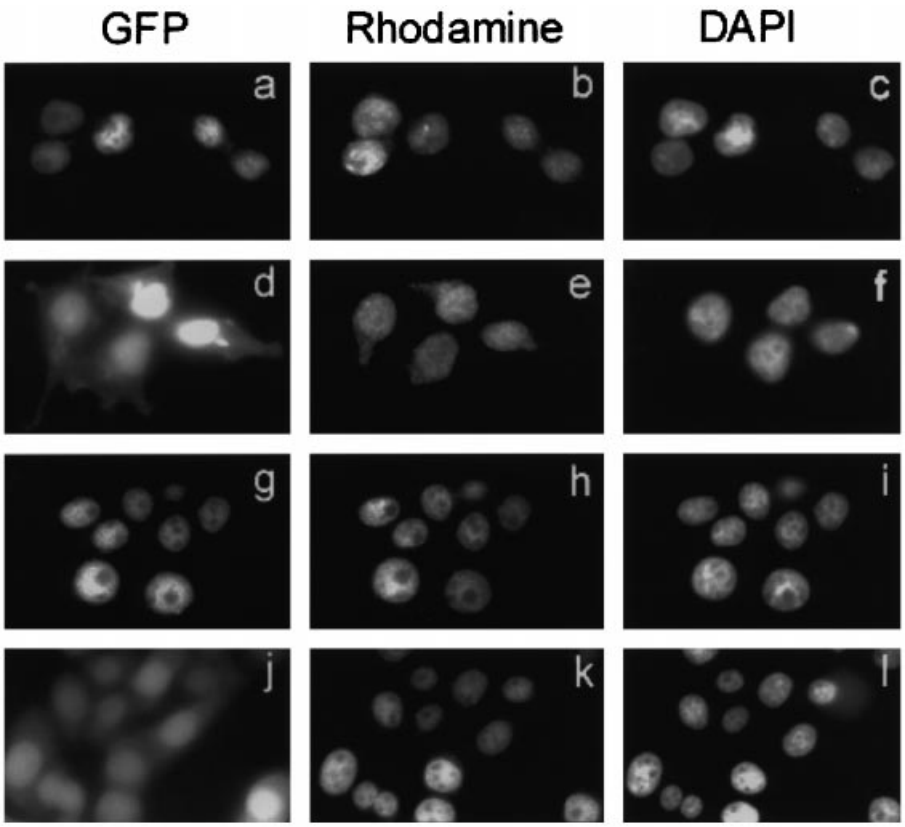

Figure 4 Co-localization of EGGAS41 and native TACC1 in HEK-293 and MDA-MB-468 cells during interphase

Full-length GAS41 fused to GFP is predominantly expressed in the nucleoplasm of stably transfected HEK-293 and MDA-MD-468 cells (**a** and **g** respectively). Indirect immunofluoresence using the TACC1 antibody and a rhodamine-labelled secondary antibody also shows that TACC1 is predominantly localized to the nuclei of the same cells (**b** and **h**). Nuclear accumulation of TACC1 is independent of the expression of a GFP fusion protein, since HEK-293 and MDA-MB-468 cells expressing GFP alone (in the cytoplasm and nucleus of transfected cells; **d** and **j**), also show predominantly nuclear expression of TACC1 (**e** and **k** respectively). Nuclei were counterstained with DAPI (**c**, **f**, **i** and **l**).

TACC3 are expressed at high levels in human cancer cell lines. Similarly, ch-TOG is overexpressed in colonic and hepatic tumours, relative to normal tissues [13], and GAS41/NuBI1 is both amplified and overexpressed at early stages of glioblastoma tumorigenesis [14]. Conversely, TACC2 has been shown to be down-regulated in a model for breast tumour progression [5]. Therefore regulation of the interaction between TACC, ch-TOG and/or GAS41/NuBI1 proteins may be critically important to the control of division of tumour cells derived from different origins.

During interphase, the human TACC proteins are found at low levels within the cytoplasm, with an increased accumulation of TACC1 and TACC3 in the nuclei of most cells within the cell population (Figure 4) [7]. Upon entering mitosis, the TACC proteins begin to associate with the centrosome and the mitotic spindle, although the interaction between TACC proteins and microtubules appears to be indirect, requiring the presence of another protein [7]. The ch-TOG protein fulfils this role in that it is able to interact directly with microtubules [15]. Interestingly, the clones that we identified corresponded to the C-terminal 529 amino acids of ch-TOG. This region contains the tubulin dimer-binding domain, required for microtubule nucleation [15]. Thus, by binding to the C-terminus of ch-TOG, TACC1 could either compete with, or stabilize, the binding of the tubulin dimer to ch-TOG. Alternatively, through its interaction with ch-TOG, TACC1 could bring regulatory proteins into the vicinity of the growing microtubule. Recently, the D-TACC protein was shown to bind to the *Drosophila* ch-TOG homologue, msps [8,9]. Furthermore, analysis of the msps and D-TACC proteins suggested that, through this interaction, D-TACC could be involved in anchoring and stabilizing microtubules to the centrosome [8,9]. This model has also been proposed for one of the functions of human TACC proteins associated with microtubules [9].

The second TACIP that we identified was the putative transcription factor GAS41/NuBI1. This protein binds *in vitro* to the nuclear matrix component NuMA, which is itself important in the assembly of the mitotic spindle. Similarly to TACC proteins, NuMA forms large oligomeric structures when overexpressed in human cells [7,16]. However, we have not been able to co-immunoprecipitate TACC1 with NuMA (results not shown), and NuMA polymers do not contain a detectable level of TACC1 protein [7], indicating that NuMA and TACC1 are found in distinct complexes. As NuMA only interacts weakly with GAS41/NuBI1 in mammalian cells [10], TACC1 may be the preferential GAS41/NuBI1 binding partner in interphase cells.

GAS41/NuBI1 is a highly conserved protein with homologues in vertebrates, invertebrates, plants and fungi [10]. The degree of homology between the human and *Drosophila* proteins (61% identity and 70% overall similarity) raises the possibility that D-TACC may also bind the *Drosophila* GAS41/NuBI1 counterpart. However, the GAS41/NuBI1 binding site of TACC1 is not conserved in the D-TACC protein, indicating that other serine/

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acidic-rich regions may act as *Drosophila* GAS41/NuBI1 binding sites. Of evolutionary importance is the fact that, to date, no TACC homologue has been detected in either plants or yeast, suggesting that the TACC–GAS41/NuBI1 interaction plays a unique role in animal biology.

Sequence analysis has indicated that the GAS41/NuBI1 protein is related to AF-9 and ENL ('acute lymphoblastic leukaemia 1 fused gene from chromosome 9' and 'eleven-nineteen leukaemia gene'), which are putative transcription factors rearranged in some acute leukaemias [14]. The region of homology between these proteins is related to the general transcription initiation factor Tfg3/TAF30/Anc1p ('transcription factor G 30 kDa subunit/TATA-binding protein associated factor 30 kDa subunit/actin non-complementing protein 1') [17]. This protein is an essential component for basal transcription and plays an important role in mediating interactions between sequence-specific transcription factors and the RNA polymerase II transcriptional machinery [18]. This suggests that GAS41/NuBI1 is involved in regulating gene transcription through a direct interaction with the basal transcription initiation complex. The predominantly nuclear localization of GAS41/NuBI1 also partially supports this idea (Figure 4) [10,19]. However, to date, GAS41/NuBI1 has not been shown to bind directly to a known transcription factor, suggesting that an accessory protein may be required for GAS41/NuBI1 to bind to DNA-sequence-specific transcription factors.

We have demonstrated that native TACC1 and TACC3 show enhanced accumulation in the nuclei of cells in culture (Figure 4), [7]. Nuclear accumulation of TACC2 also increases in human microvascular endothelial cells in response to treatment with erythropoietin [20]. This suggests that TACC proteins may play a role in signal transduction to the nucleus in response to certain cytokines. The murine Tacc3 protein, AINT, has recently been shown to interact via the TACC domain with the ARNT transcription factors [4]. Thus it is tempting to speculate that TACC proteins may be involved in the final stages of signal transduction in the nucleus, providing a structural link enhancing the binding of DNA-sequence-specific transcription factors, such as ARNT, to a GAS41/NuBI1-containing basic transcription factor complex in the nucleus. This hypothesis is further supported by the observation that overexpression of Tacc3 can enhance the ARNT-mediated hypoxic induction of the erythropoietin promoter, and can also increase the activation of a xenobiotic response element–luciferase reporter by dioxin [4]. Dysregulation of TACC and GAS41/NuBI1 proteins may therefore contribute to tumorigenesis by altering the transcriptional response to cell signalling pathways.

In conclusion, it appears that the TACC proteins can form multiple different protein complexes in the cell. In the cytoplasm, TACC proteins appear to act as scaffolding/bridging proteins important for centrosomal function. The TACC proteins may perform a similar function in the nuclear matrix, by acting as scaffolding or bridging proteins between transcription factors and basal transcription initiation complexes. This potential transcriptional regulatory role of the TACC proteins may be particularly important in tumorigenesis, and now represents a new avenue of TACC research that needs to be addressed.

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